

## CHANGES IN THE DENTAL PULP CALCIFICATIONS WITH A LAMELLAR CONCENTRIC STRUCTURE AND CALCIFICATIONS WITH AN AMORPHOUS TO FINELY GRANULAR STRUCTURE

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### Abstract

The aim of the study was to inspect change in the pulp with dental calcifications by different structure.

We carried out the research on 40 pulps after indicated tooth extraction, during which we made a vertical section of the teeth and removed the pulps, and 60 extirpated pulps from teeth with an endodontic diagnosis of pulpitis chronica.

With the method of light microscopy, and by using standard differential histo-chemical coloring, results were obtained on the Institute of Pathology of the Faculty of Medicine in Skopje.

Results: 2 morphological images of changed pulp were obtained with the method of light microscopy with the application of standard differential histochemical staining: dental pulp with calcifications with a lamellar concentric structure; dental pulp with calcifications present with an amorphous to finely granular structure. In both groups, a reduction of cellularity in the connective tissue is observed, with evident but gradual hyalinization, which takes the form of wider areas or tracks, dissociated from relatively preserved loose connective tissue.

Pulps containing nodular calcifications show a certain degree of congestion of blood vessels, in contrast to dispersed and irregularly shaped calcifications.

**Key words:** dental pulp, dental calcifications, histopathological analysis.

### Introduction

Calcifications in the dental pulp, as a phenomenon with diverse occurrence and manifestation, represent a subject of constant interest not only from the perspective of being a separate dental entity but also because they are interesting to observe and deal with from diagnostic and therapeutic aspect.

The fact that they are referred to as being provocateurs of pain with different intensity makes them cause difficulties in diagnosing.

When observed from therapeutic aspect, they appear to be of greater importance because they can make the access to the dental roots difficult or in some cases completely impossible, and they can also be the reason for groundless extraction of a tooth or a group of teeth.

A greater percentage of denticles are found in maxillary teeth compared to the mandibular teeth, except for incisors, which are more common in the lower jaw [1,2,3,4,5,6,7].

The comparison of teeth that are carious or restored, in relation to intact teeth, shows that denticles are more often found in carious, i.e., restored teeth, and less often in non-restored teeth [8].

The percentage of representation of denticles according to gender shows that they are more represented in the male sex, compared to the percentage of representation in the female sex, in both jaws [9,10].

Calcifications with a lamellar-concentric structure are spherical in shape. They are nodules, stain more intensely with hematoxylin, show more intense basophilia, unlike denticles. The deposition of calcium salts is rough, with the absence of fine trabecular-tubular structures visible as in denticles [2,11,12].

Calcifications with granulated fine granular structure, according to their shape, were spherical and oval, or irregular.

These calcifications had similar structure to previous, presenting from zones of encrustations which were amorphous and uniformed, up to zones with fine granulated material [2,11,12]. There are variations of pulp mineralization, either in structure, morphology, dimensions, or location [13].

In the root canal space, they present a more diffuse structure, with tubular or cylindrical configuration, partially following the root canal design. The size varies from microscopic bodies to structures occupying the whole pulp chamber space [14].

### **Material and methods**

Material for histological examination was provided with endodontic extirpation and vertical section after tooth extraction; the material consisted of 40 extirpated vital pulps and 60 extirpated pulps of teeth with endodontic diagnosis of chronic pulpitis.

Teeth were being cured in endodontic manner up to their final obturation. For histological processing, various methods and procedures were used, such as: fixation, decalcification, tissue processing, provision of paraffin sections, standard coloring, differential coloring, microscopy, and morphological analysis with photographs.

Fixation, as the most important method in stabilizing proteins and preserving nuclei and cell structures, is performed with an aqueous solution of formaldehyde. All tissue samples were immersed in buffered 10% formalin, i.e. 4% aqueous formaldehyde solution. The volume ratio of formalin to tissue material was at least 10:1. The shortest duration of fixation was not less than 48 hours.

Necessary for obtaining paraffin tissue molds is the softening of the tissue through a decalcification process. The decalcification took place according to a combined technique of fast and slow decalcification.

As a strong decalcinate in the accelerated decalcification procedure, a 10% aqueous solution of nitric acid was used, with the following composition:

|                 |        |
|-----------------|--------|
| Formalin        | 10 ml. |
| Distilled water | 80 ml. |
| Nitric acid     | 10 ml. |

In the spore and controlled decalcification procedure, two different solutions were used: 3% aqueous solution of nitric acid and 5% aqueous solution of formic acid, with the following composition:

|                          |                          |
|--------------------------|--------------------------|
| Nitric acid decalcinate: | Formic acid decalcinate: |
| Formalin 15 ml.          | Formalin 15 ml.          |
| Distilled water 82 ml.   | Distilled water 80 ml.   |
| Nitric acid 3 ml.        | Formic acid 5 ml.        |

After the fast and slow decalcification method, the material from the extracted teeth was processed, in a time duration of 2 to 12 months. The dental pulps were treated by the spore-controlled

decalcification method for a duration of 30 min. up to 150 min. Half of the dental pulp material was processed in a delayed decalcification procedure.

First, standard tissue sections were obtained, then the tissue sample was rehydrated and returned to decalcinate with the same decalcification time as the slow and controlled decalcification procedure described above.

Tissue processing was performed in a Shandon brand Citadela 2000 tissue processor using the common and abbreviated procedure method.

The extracted teeth were processed with the usual procedure, and the extirpated pulps, due to the small volume of the material and the possibility of tissue dissolution, were processed according to the shortened method, which corresponds in practice and is known as "manual guidance of the material".

The paraffin sections were obtained with a "Leica" and "Reinhard" sliding microtome. The thickness of the sections was between 4 and 5 microns, with a longitudinal cutting direction, i.e., parallel to the largest diameter of the pulp and the tooth.

### **Microscopic morphological analysis**

For hardware and software in this segment, the dedicated modification of the Image analyzer Lucia M was used. During the morphological analysis, the following morphological parameters were taken - size of the calcifications, shape, relation to the surrounding tissue from the pulp and cellular substrate of the pulp.

### **Results**

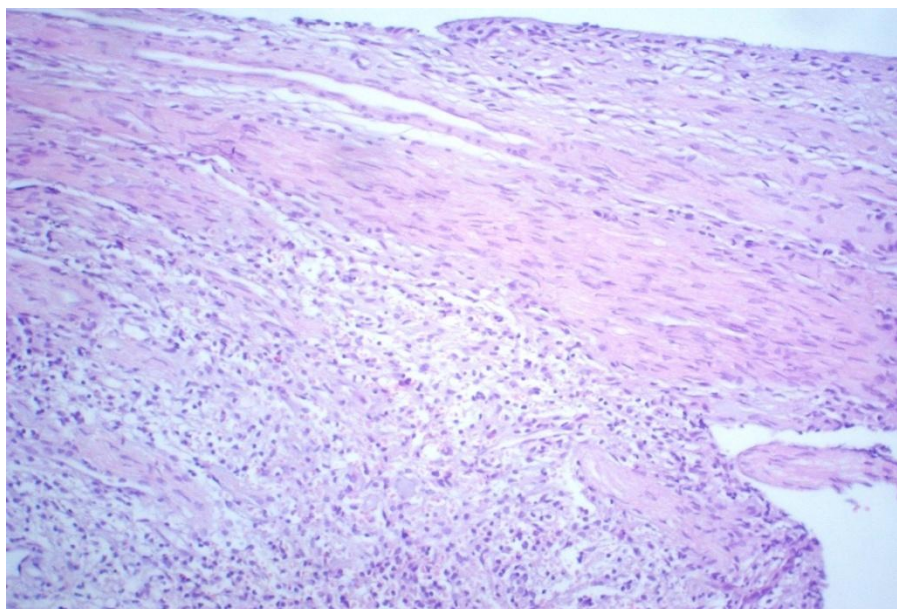
According to method of light microscopy, and by using standard differential histo-chemical coloring, 2 morphological images of changed pulp were obtained: (1) dental pulp with calcifications with a lamellar concentric structure; and (2) dental pulp with calcifications present with an amorphous to finely granular structure.

In both groups, a reduction of cellularity in the connective tissue is observed, with evident but gradual hyalinization, which takes the form of wider areas or tracks, dissociated from relatively preserved loose connective tissue. Pulps containing nodular calcifications show a certain degree of congestion of blood vessels, in contrast to dispersed and irregularly shaped calcifications.

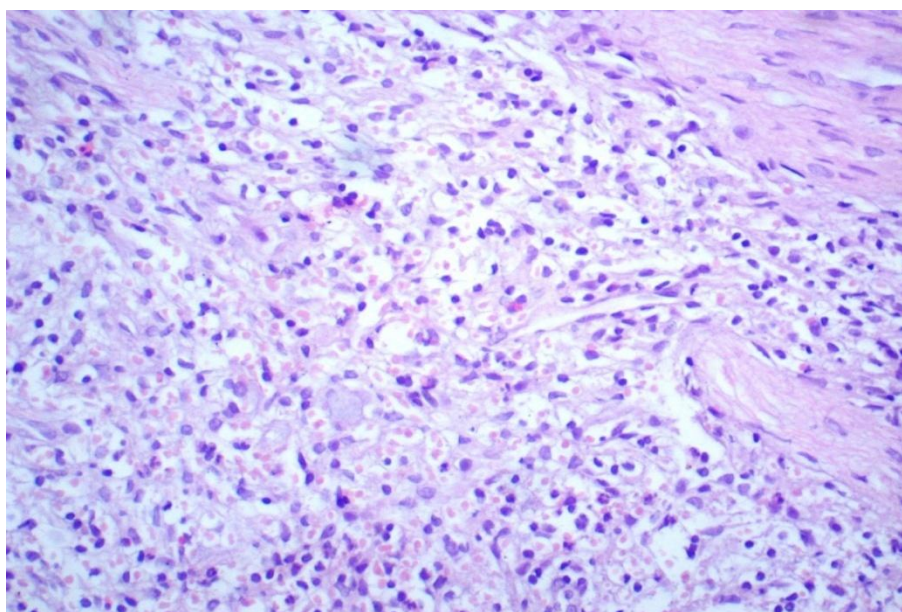
Irregular-shaped calcifications, as well as dispersed fine-grained ones, are more associated with middle and older age, and the presence of hyaline-transformed, but not congestively-changed pulp, indicates basically senile, degenerative changes of the pulp with or without adjuvant agents in the formation of calculus (Figs 1,2,3,4).

In younger individuals, this pulp morphology, associated with congested blood vessels, can be considered as post-inflammatory residue or a late phase of a protracted immunologically modified inflammatory process, with altered micro homeostatic and hemodynamic parameters.

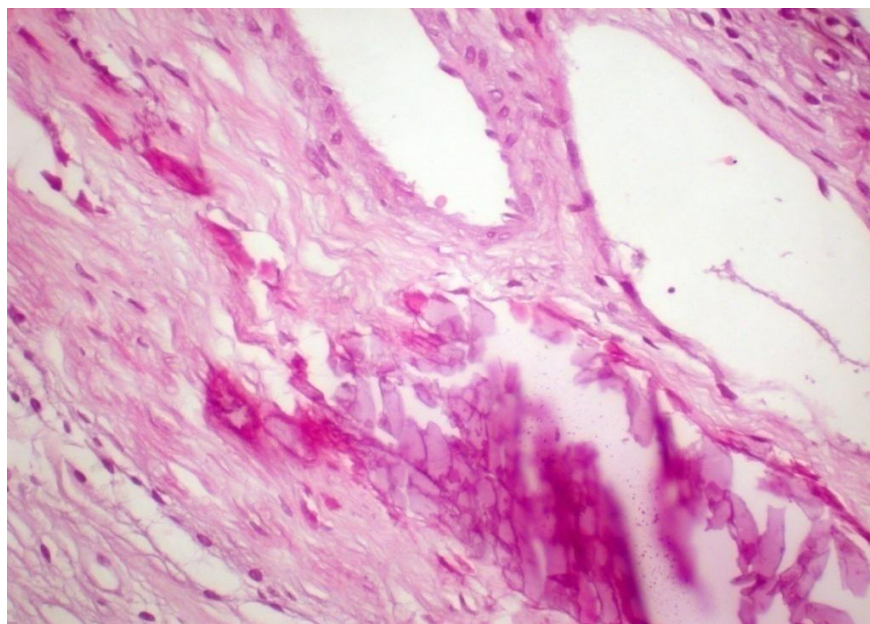
No pericalcification odontoblastic transformation or proliferation was observed in any of the examined case samples.



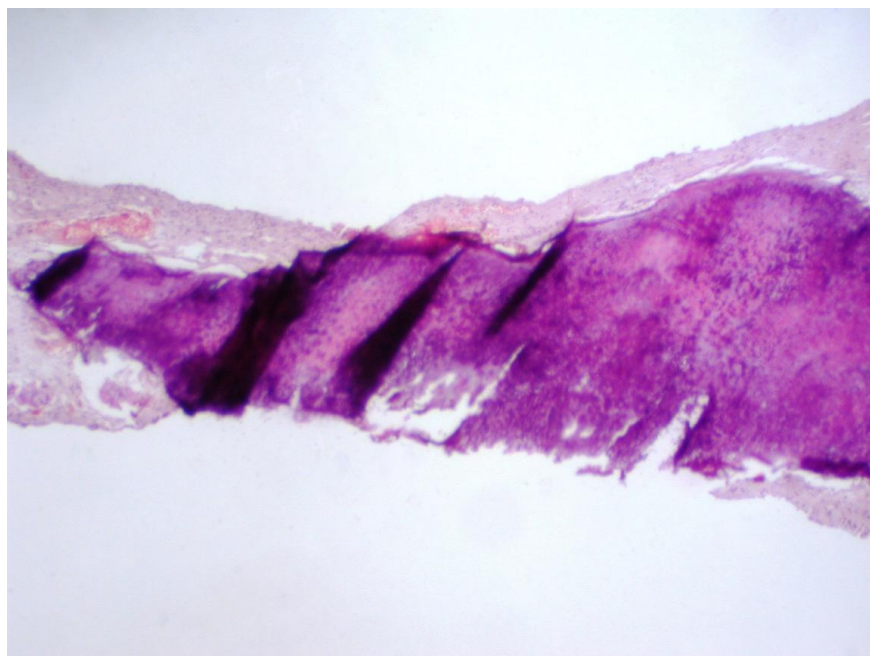
**Figure 1.** Staining by HE, magnification 10 X 10, environment of non-dentine calculus, in the pulp there is an abscessing phlegmonous inflammatory infiltrate of mixed cellularity that partially layers the bundles of the connective tissue of the pulp.



**Figure 2.** Staining by HE, magnification 10 X 40, environment of non-dentine calculus, in the pulp there is an abscessing phlegmonous inflammatory infiltrate of mixed cellularity, which partially layers the bundles of the connective tissue of the pulp.



**Figure 3.** HE staining, magnification 10 X 20, non-dentine, irregularly shaped amorphous crystalline calculus with surrounding hyalinized pulp and wide free vascular spaces.



**Figure 4.** HE staining, magnification 10 X4, a formation of non-dentine calculus with a fine granular structure, in size it occupies almost the entire length and width of the pulp. Noticeable congested blood vessels.



## **Discussion**

As a basis for discussion is the finding that the dental calcifications represent a separate model of pathological calcification, fitting into overall pathological classification, although with different structure. Literature is rich with descriptions of dental calcifications.

The greatest attention is paid to the prevalence of denticles [15,16,17,18,19].

In search of associative morphological comparisons, the section of these calculi to a significant extent reminded of the tree circles. The lamellar structure and concentricity of the discolorations of the transverse sections of these calculi imply that there was organic matrix, as initial nidus, with timely protracted circular, organically interpolated incrustation with calcium salts.

These morphological changes, known as false denticles, fall under the wider group of dystrophic calcifications and as being such, from terminological perspective, they probably deserve to be referred to with another name.

Common to all calcifications from the group of calcifications with a granular fine-grained structure is the intense restaining with hematoxylin, i.e. basophilic coloring, which indicates the greatest presence of calcium salts, compared to the previous group.

The organic matrix is maximally reduced, so that after the decalcification stage, it is transparent and missing in places, forming empty fissures or lacunar spaces. This type of uniform calcification indicates continuous temporal dynamics in the deposition of calcium in a short time interval. Post-necrotic changes, with appropriately changed homeostatic mechanisms, are a prerequisite for the formation of this type of calcifications.

The similarity of the morphological changes of the pulp in these two groups, as well as the need for larger series of examined material, does not leave space for the morphology of the observed changes to be considered from the aspect of morphogenetic events, i.e., what is the development of pathogenetic mechanisms.

When observed from therapeutic aspect, they appear to be of greater importance because they can make the access to the dental roots difficult or in some cases completely impossible, and they can also be the reason for groundless extraction of a tooth or a group of teeth.

The results obtained from the carried out examinations showed that the histopathological analysis led to the following conclusions: The calcifications in the dental pulp are dentinal and non-dentinal: - the dentinal calcifications are spherical, nodular, solitary and more numerous, they contain greater amount of organic matrix, they occur at early age and have hamartomatous aspect; - the non-dentinal calcifications could be nodularly spherical, irregular in shape ranging to them representing punctiform encrustations.

They contain smaller amount of organic matrix, occurred in the middle or older age and have inflammatory dystrophic background [20].

The topography of the nodules showed heterogeneous relief, revealing smooth and compact areas contrasting with the rugged and porous ones. The chemical composition varied depending on the location of the nodule in the pulp cavity and the relief of the analyzed area. Radicular stones presented considerably lower calcium and phosphorus content than coronary nodules [11,12,13].

## **Conclusion**

The structural characteristics of the pulp with dental calcification, described in our study, showed a reduction of cellularity in the connective tissue, with gradational hyalinization, with loose connective tissue. Pulp containing nodular calcifications show a certain degree of congestion of blood vessels, in contrast to dispersed and irregularly shaped calcifications. In none of the examined cases, pericalcification odontoblastic transformation or proliferation was observed.

With our study, we verified the histopathological changes in the radicular part of the pulp, in teeth with the presence of denticles. We now know what happens to the surrounding pulp tissue. From a therapeutic point of view, if the suspect tooth is asymptomatic, there is no need to devitalize it.

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